THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 272, No. 20, Issue of May 16, pp. 12984–12988, 1997

Printed in U.S.A.

Secretin Promotes Osmotic Water Transport in Rat Cholangiocytes by Increasing Aquaporin-1 Water Channels in Plasma Membrane

EVIDENCE FOR A SECRETIN-INDUCED VESICULAR TRANSLOCATION OF AQUAPORIN-1*

(Received for publication, December 24, 1996, and in revised form, March 6, 1997)

Raul A. Marinelli‡, Linh Pham‡, Peter Agre§, and Nicholas F. LaRusso‡¶

From the ‡Center for Basic Research in Digestive Diseases, Departments of Internal Medicine, Biochemistry, and Molecular Biology, Mayo Clinic and Foundation, Mayo Medical School, Rochester, Minnesota 55905 and the §Departments of Biological Chemistry and Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Although secretin is known to stimulate ductal bile secretion by directly interacting with cholangiocytes, the precise cellular mechanisms accounting for this choleretic effect are unknown. We have previously shown that secretin stimulates exocytosis in cholangiocytes and that these cells transport water mainly via the water channel aquaporin-1 (AQP1). In this study, we tested the hypothesis that secretin promotes osmotic water movement in cholangiocytes by inducing the exocytic insertion of AQP1 into plasma membranes. Exposure of highly purified isolated rat cholangiocytes to secretin caused significant, dose-dependent increases in osmotic membrane water permeability (P_f) (*e.g.* increased by 60% with 10^{-7} M secretin), which was reversibly inhibited by the water channel blocker $HgCl_2$. Immunoblotting analysis of cholangiocyte membrane fractions showed that secretin caused up to a 3-fold increase in the amount of AQP1 in plasma membranes and a proportional decrease in the amount of the water channel in microsomes, suggesting a secretin-induced redistribution of AQP1 from intracellular to plasma membranes. Both the secretin-induced increase in cholangiocyte P_f and AQP1 redistribution were blocked by two perturbations that inhibit secretin-stimulated exocytosis in cholangiocytes, *i.e.* treatment with colchicine and exposure at low temperatures (20 and 4 °C). Our results demonstrate that secretin increases AQP1-mediated P_f in cholangiocytes. Moreover, our studies implicate the microtubule-dependent vesicular translocation of AQP1 water channels to the plasma membrane, a mechanism that appears to be essential for secretin-induced ductal bile secretion and suggests that AQP1 can be regulated by membrane trafficking.

Bile is formed primarily by hepatocytes and secreted at the bile canaliculus; subsequently, its volume and composition are modified in the lumen of bile ducts as a result of the transport of water and solutes by cholangiocytes (1, 2). While this ductal bile secretion results from the osmotically driven movement of water, the regulatory and mechanistic aspects are obscure. We recently reported that cholangiocytes (unlike hepatocytes) express the water-selective channel protein aquaporin-1 (AQP1)[1] and proposed that ductal bile secretion results from the movement of water across this protein (3, 4). Based on studies in renal epithelial cells, it is currently thought that AQP1 is constitutively inserted into plasma membranes and is not hormone responsive (5, 6).

Secretin is known to stimulate ductal bile secretion via specific receptors on cholangiocytes (7). We and others recently proposed that secretin-induced bile secretion was associated with the microtubule-dependent exocytic insertion of cytoplasmic vesicles into the cholangiocyte plasma membrane (8–10). Interestingly, hormone-regulated exocytic movement of transporters has been demonstrated in other cell types (11). For example, in renal collecting tubule cells the water channel aquaporin-2 moves to and from the apical plasma membrane in the presence and absence of vasopressin, respectively (12). For these reasons, we hypothesized that secretin stimulates ductal bile secretion by inducing the translocation of functional AQP1 water channels into the plasma membrane of cholangiocytes.

MATERIALS AND METHODS

Preparation and Incubation of Cholangiocytes—Cholangiocytes (>95% pure) were isolated from livers of male Fischer rats by enzymatic digestion and mechanical disruption and then immunopurified using Dynabeads M-450 and collected with a magnet as described previously (13). In colchicine and low temperature studies, cholangiocytes were prepared from rats 3 weeks after bile duct ligation, a maneuver that induces selective proliferation of cholangiocytes and thus generates an increased number of cells available for experiments. These cholangiocytes retain normal phenotypic features (14) and respond to secretin in a manner similar to cholangiocytes from normal rats (15). In all experiments, cell viability was greater than 90% as assessed by trypan blue exclusion. All incubations were carried out in Krebs-Ringer-HEPES buffer, pH 7.4.

Following isolation, cells were incubated according to one of three protocols: (*a*) for 15 min at 37 °C in the presence of 0–10^{-6} M secretin (Peninsula Laboratories, Belmont, CA); (*b*) for 1 h at 37 °C in the presence of 50 μM colchicine or lumicolchicine (Sigma) and then for an additional 15 min in the presence of 0 or 10^{-7} M secretin; (*c*) for 30 min at 37, 20, or 4 °C and then for an additional 15 min in the presence of 0 or 10^{-7} M secretin at these temperatures.

Osmotic Water Transport Studies—Following incubation as described above, cells were washed and suspended in cold, 300 mosMKrebs-Ringer-HEPES buffer, pH 7.4, without $CaCl_2$. We have previously reported that osmotic water transport in cholangiocytes is not significantly affected at low temperature (3). Therefore, in this work

* This work was supported by National Institutes of Health Grants DK 24031, HL 33991, HL 48268, and a research fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina) (to R. A. M.). A preliminary report of part of this work was presented at the 1996 Meeting of the American Association for the Study of Liver Diseases, Chicago, IL (Marinelli, R. A., Pham, L., Agre, P., and LaRusso, N. F. (1996) *Hepatology* **24,** 355A). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Center for Basic Research in Digestive Diseases, Mayo Clinic, 200 First St. SW, Rochester, MN 55905. Tel.: 507-284-1006; Fax: 507-284-0762; E-mail: larusso.nicholas@Mayo.edu.

[1] The abbreviations used are: AQP1, aquaporin-1; P_f, membrane water permeability.

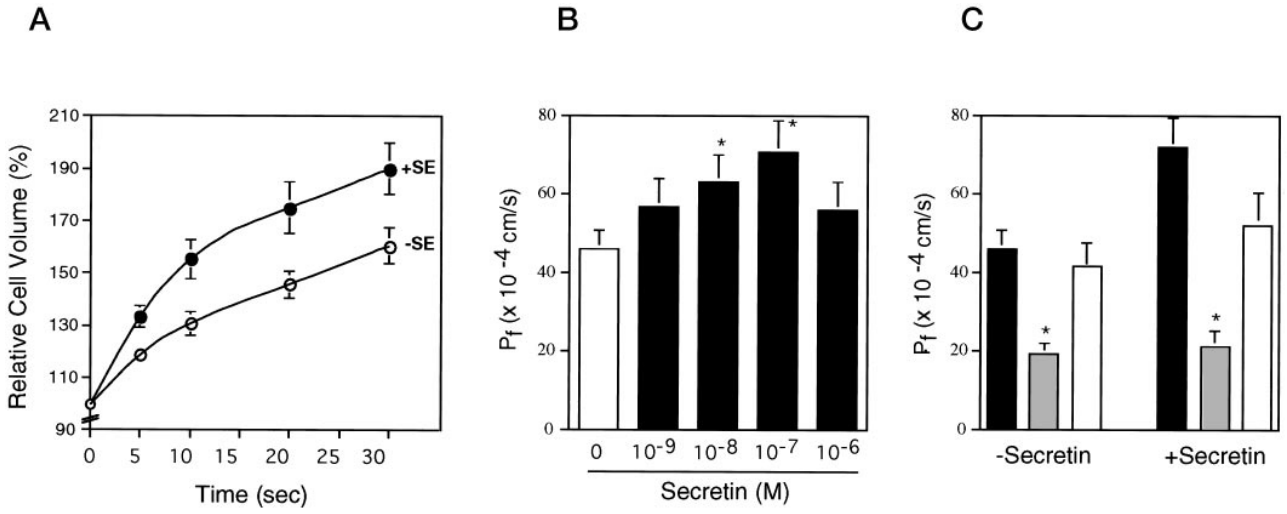

FIG. 1. **Effect of secretin (*SE*) on the osmotic water transport in isolated rat cholangiocytes.** *A*, time course of the osmotic swelling of cholangiocytes. Cells were incubated at 37 °C for 15 min in the presence of 0 or 10^{-7} M secretin, cooled at 4 °C, and exposed to 30 mosM hypotonic buffer at time zero. *B*, membrane water permeability (P_f) of cholangiocytes incubated in the presence of 0–10^{-6} M secretin at 37 °C for 15 min. P_f was calculated from data of cholangiocyte swelling in 30 mosM buffer. *, $p < 0.05$ compared with 0 M secretin (Student's *t* test). *C*, effect of $HgCl_2$ on the secretin-induced increase in cholangiocyte P_f. Cells were incubated at 37 °C for 15 min in the presence of 0 or 10^{-7} M secretin and cooled at 4 °C. Before the water transport assay, they received no further treatment, or they were treated with 0.3 mM $HgCl_2$ for 5 min or 0.3 mM $HgCl_2$ for 5 min followed by 10 min with 5 mM 2-mercaptoethanol. *, $p < 0.05$ compared with $-HgCl_2$ or $+HgCl_2$ + me (Student's *t* test). *Black bar*, $-HgCl_2$; *gray bar*, $+HgCl_2$; *white bar*, $HgCl_2$ + 2-mercaptoethanol. All the data shown are mean ± S.E. of measurements from 15 to 62 cholangiocytes in each experimental group.

water transport studies were performed at 4 °C to prevent exo- and endocytic events in cholangiocytes (10) that could potentially modify a secretin-induced subcellular relocation of AQP1. The size of cholangiocytes was determined by quantitative phase contrast microscopy, a methodology previously validated (3, 4). Briefly, serial photographs of the same group of cells placed on coverslips coated with polylysine in isotonic (300 mosM) and hypotonic media (30 mosM) were digitized, and cell diameters were measured with an image analysis software program (ANALYZE™, Mayo Foundation). Cell volumes were estimated based on the spherical shape of cholangiocytes using 4.5-μm immunomagnetic beads as internal standards. The osmotic membrane water permeability (P_f) was calculated from the initial rate of cell swelling as described previously (3).

In some experiments, cholangiocytes were incubated with the known water channel blocker, $HgCl_2$, for 5 min or with $HgCl_2$ followed by 10 min with 2-mercaptoethanol before measuring water permeability; our previous work had shown that $HgCl_2$ at the concentration used (0.3 mM) was not toxic for cholangiocytes (3).

Preparation of Subcellular Membrane Fractions—Plasma and microsomal membrane fractions were prepared from the incubated cells by differential centrifugation. Briefly, cholangiocytes were washed and sonicated in 0.3 M sucrose containing 0.01% soybean trypsin inhibitor, 0.1 mM phenylmethanesulfonyl fluoride, and 0.1 mM leupeptin (Sigma). The immunomagnetic beads were separated using a magnet.

The plasma membrane fraction was obtained by centrifugation at 200,000 × *g* for 60 min on a discontinuous 1.3 M sucrose gradient as described previously (16). After removing the plasma membrane band, the sucrose gradient was sonicated, diluted to 0.3 M, and centrifuged at 17,000 × *g* for 30 min. The pellet obtained was designated "remaining intracellular membrane fraction," and the resulting supernatant was centrifuged at 200,000 × *g* for 60 min to yield the microsomal membrane fraction.

Protein concentration was determined by the fluorescamine method using bovine serum albumin as standard (17). Alkaline phosphatase activity (a plasma membrane marker) was assessed using a commercially available enzyme kit (Sigma). Microsomal esterase activity (a marker for the endoplasmic reticulum) was measured by the method of Beaufay and Berthet (18).

Immunoblotting for AQP1—Solubilized cholangiocyte membrane fractions were subjected to SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose sheets. After blocking, blots were incubated overnight at 4 °C with AQP1 antiserum (19) diluted 1:500. The blots were then washed and incubated with horseradish peroxidase-conjugated goat antirabbit immunoglobulin (Tago, Inc., Burlingame, CA), and bands were detected by the enhanced chemiluminescence detection system (ECL, Amersham). Autoradiographs were obtained by exposing nitrocellulose sheets to Kodak XAR film, and the bands were quantitated by laser densitometry.

RESULTS

Effect of Secretin on the Osmotic Water Transport in Cholangiocytes—The time course of relative cholangiocyte volume in response to an outwardly directed NaCl gradient is shown in Fig. 1*A*. The osmotic gradient caused water influx and cell swelling. The rate of the swelling response was significantly increased by 10^{-7} M secretin treatment. P_f values of cholangiocytes treated with several doses of secretin are summarized in Fig. 1*B*. The effect of secretin on cholangiocyte P_f was dose-dependent, and P_f increased with increasing concentrations of secretin up to 10^{-7} M (~60%); 10^{-6} M failed to further stimulate P_f. Average cholangiocyte volume (in isotonic media) was not affected by any of the doses of secretin used. As shown in Fig. 1*C*, the 10^{-7} M secretin-induced increase of cholangiocyte P_f was inhibited by the known water channel blocker, $HgCl_2$, and was restored with the sulfhydryl reagent 2-mercaptoethanol (20). Similar results were seen with 10^{-8}–10^{-6} M secretin (data not shown).

Together these data suggest that secretin promotes osmotic water transport in cholangiocytes via a mechanism mediated by mercury-sensitive water channels.

Effect of Secretin on the Distribution of AQP1 in Cholangiocytes—To investigate whether secretin increased cholangiocyte P_f by inducing the translocation of the mercury-sensitive water channel AQP1 from subcellular organelles to cell surface, we performed quantitative immunoblotting of cholangiocyte intracellular and plasma membranes. AQP1 protein was mostly recovered in cholangiocyte plasma and microsomal membranes, with only negligible amounts of AQP1 present in the remaining intracellular membrane fraction (see "Preparation of Subcellular Membrane Fractions" under "Materials and Methods"). Exposure of cells to secretin resulted in an increase of AQP1 in cholangiocyte plasma membranes by (192%, $p < 0.01$) and a simultaneous decrease of AQP1 in microsomes (56%, $p < 0.01$) (Fig. 2). Secretin did not alter either the yields of total membrane protein, the specific activity of microsomal esterase, or plasma membrane alkaline phosphatase in the

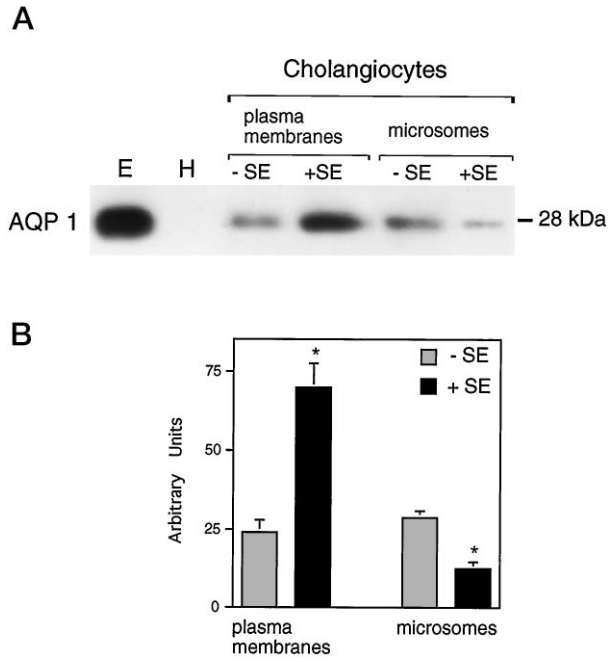

FIG. 2. **Effect of secretin (*SE*) on the amount of AQP1 protein in cholangiocyte membranes.** *A*, representative immunoblot for AQP1 on plasma and microsomal membrane fractions. Cells were incubated in the absence (−) or presence (+) of 10^{-7} M secretin, and cholangiocyte membrane fractions were prepared as described under "Materials and Methods." *E*, erythrocyte plasma membranes (positive control); *H*, hepatocyte plasma membranes (negative control). Each *lane* was loaded with 10 μg of protein for cholangiocyte membrane fractions and hepatocyte plasma membranes and 1 μg for erythrocyte plasma membranes. *B*, densitometric analysis of four separate experiments expressed in arbitrary units as mean ± S.E. *, $p < 0.01$ for secretin effect (Student's *t* test).

cholangiocyte plasma and microsomal membrane fractions (data not shown).

These data are consistent with a secretin-induced relocation (presumably via vesicles) of AQP1 from intracellular to plasma membranes.

Effect of Perturbations That Disrupt Vesicular Transport—To provide support for our interpretation that secretin-induced AQP1 redistribution in cholangiocytes involves a vesicular transport mechanism, we evaluated the effect of two perturbations reported by us to disturb secretin-induced exocytosis in cholangiocytes, *i.e.* treatment with the microtubule blocker colchicine and exposure at low temperature (10).

Pretreatment of cholangiocytes with colchicine (but not with its inactive analog β-lumicolchicine), as well as incubation at 20 and 4 °C, markedly inhibited the increase in plasma membranes and the decrease in microsomes of AQP1 protein induced by secretin (see Fig. 3, *A* and *B*, and Fig. 4, *A* and *B*). These two perturbations also selectively blocked the secretin-induced increase in cholangiocyte P_f (Figs. 3*C* and 4*C*).

Together, these data suggest that secretin promotes osmotic water transport in cholangiocytes by inducing the temperature- and microtubule-dependent vesicular translocation of AQP1 from an intracellular compartment to plasma membrane.

DISCUSSION

Current concepts concerning hormonal regulation of cell membrane water permeability come primarily from studies of the kidney. In the renal collecting duct, vasopressin binds to its receptor on the basolateral membrane of tubular principal cells; the intracellular levels of cyclic AMP rise; intracellular vesicles containing AQP2 water channels fuse with the apical membrane; and water transport increases (21, 22). In contrast, the homologous water channel AQP1 is thought to be constitutively expressed in plasma membranes of renal cells (*i.e.* in proximal tubules and the descending limbs of Henle) and other transporting epithelia (*e.g.* lung, trachea, eye, pancreas, etc.) (5, 6, 12). Thus, our study in cholangiocytes provides the first evidence for hormone-regulated membrane insertion of AQP1 water channels.

Secretin is known to stimulate ductal bile secretion by binding to its receptor on the basolateral domain of cholangiocytes (7), a ligand-receptor interaction that also activates cyclic AMP. Cyclic AMP then activates cystic fibrosis transmembrane regulator Cl^- channels, which would operate in parallel with an apical Cl^-/HCO_3^-. Although the actual solutes responsible for the osmotically driven ductal water secretion are unknown, HCO_3^- as well as Cl^- are currently considered likely to be involved (1, 2). Secretin-induced bile secretion has also been shown by morphologic techniques to be associated with a decrease in cytoplasmic vesicles (8), which was interpreted as reflecting exocytic insertion into cholangiocyte plasma membrane (10). Thus, these observations support the occurrence of an exocytic process involved in secretin-induced ductal water secretion. In line with these observations, the results of this study indicate that secretin enhances cholangiocyte P_f by inducing the exocytic insertion of AQP1 into cholangiocyte plasma membranes. Although the precise cellular mechanisms by which secretin mediates this exocytic translocation of AQP1 require additional study, they are likely to overlap with processes that modulate, via a cyclic AMP cascade, the trafficking of the vasopressin-regulated water channel AQP2 in the kidney and the insulin-sensitive glucose transporter GLUT 4 in adipocytes and muscle cells (11).

Our data demonstrate that secretin significantly increased cholangiocyte water permeability, an effect that was reversibly inhibited by the known water channel blocker $HgCl_2$. These results indicate that the mercury-sensitive water channel AQP1 mediates the secretin-induced increase in cholangiocyte P_f. Although the mercury-sensitive water channel AQP2 is not expressed in cholangiocytes,[2] we cannot exclude that other, as yet unidentified, mercury-sensitive water channels contribute to the secretin effect.

The dose-response relationship of the secretin-stimulated increase in cholangiocyte P_f showed a progressive rise up to 10^{-7} M; increasing the concentration to 10^{-6} M failed to further increase P_f (Fig. 1*B*), suggesting that autoinhibition is activated at this concentration, a phenomenon also reported for vasopressin-induced water permeability in the kidney (23).

As the functional studies suggested, secretin markedly increased the amount of AQP1 in cholangiocyte plasma membranes while simultaneously decreasing the amount of AQP1 in microsomes (Fig. 2). Based on the quantitative immunoblots for AQP1 and the total membrane proteins in each cholangiocyte membrane fraction, we estimated that under basal (non-stimulated) conditions approximately 70% of the total amount of cholangiocyte AQP1 would reside in intracellular membranes, whereas the rest would be present in plasma membranes (*i.e.* ~30%). Interestingly, in cells in which AQP1 is constitutively expressed, most of the water channel (~95%) is associated with plasma membranes (5, 6). After secretin treatment, AQP1 became predominant in plasma membranes (*i.e.* about 65% of total), and this increase was proportional to the decrease of AQP1 observed in microsomes; the total amount of AQP1 in both membrane fractions was not affected by secretin. The estimated subcellular distributions of AQP1 in basal and secretin-stimulated cholangiocytes are in very good agreement

[2] N. F. LaRusso, unpublished data.

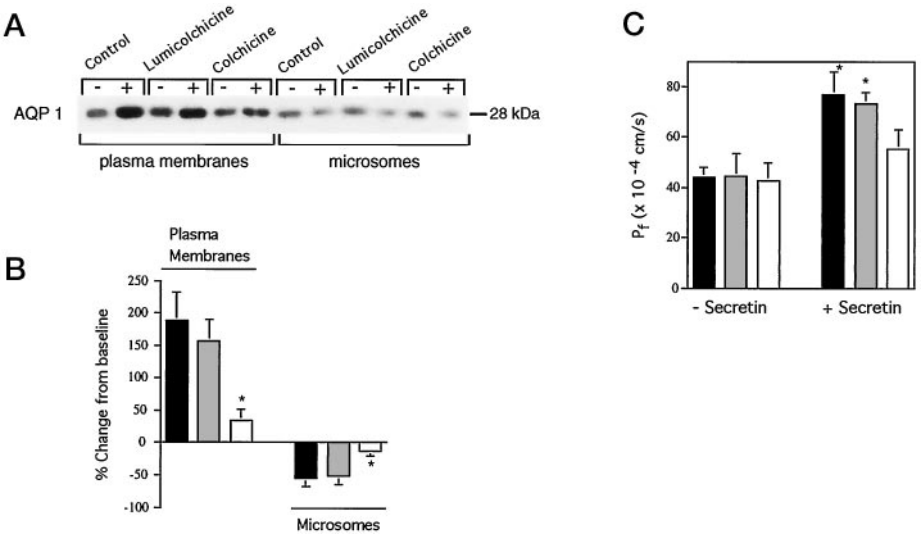

FIG. 3. **Effect of colchicine on the secretin-induced redistribution of AQP1 and the increase in cholangiocyte water permeability.** *A*, representative immunoblot for AQP1 on plasma and microsomal membrane fractions. Cells were incubated for 1 h at 37 °C in the presence of 50 μM colchicine, lumicolchicine, or vehicle and then for an additional 15 min in the absence (−) or presence (+) of 10^{-7} M secretin. Membranes were prepared as described under "Materials and Methods." Ten μg of protein were loaded in each *lane*. *B*, densitometric analysis of three separate experiments expressed as percent change induced by secretin (mean ± S.E.). *, $p < 0.05$ compared with control (Student's *t* test). *C*, membrane water permeability (P_f) of cholangiocytes treated as mentioned above. P_f was calculated from data of cholangiocyte swelling in 30 mosM buffer. *Black bar*, control; *gray bar*, lumicolchicine; *white bar*, colchicine. Data are mean ± S.E. from 15 to 35 cholangiocytes in each experimental group. *, $p < 0.05$ compared with corresponding −secretin values (Student's *t* test).

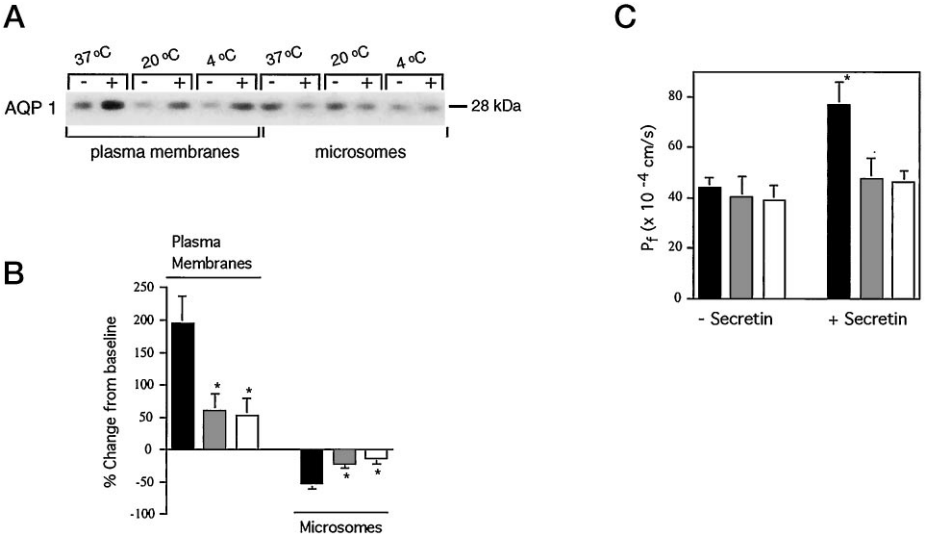

FIG. 4. **Effect of low temperature incubation on the secretin-induced redistribution of AQP1 and the increase in cholangiocyte water permeability.** *A*, representative immunoblot for AQP1 on plasma and microsomal membrane fractions. Cells were incubated at either 37, 20, or 4 °C for 30 min and then for an additional 15 min in the absence (−) or presence (+) of 10^{-7} M secretin. Membranes were prepared as described under "Materials and Methods." Ten μg of protein were loaded in each *lane*. *B*, densitometric analysis of three separate experiments expressed as percent change induced by secretin (mean ± S.E.). *, $p < 0.05$ compared with control (Student's *t* test). *C*, membrane water permeability (P_f) of cholangiocytes treated as mentioned in *panel A*. P_f was calculated from data of cholangiocyte swelling in 30 mosM buffer. *Black bar*, 37 °C; *gray bar*, 20 °C; *white bar*, 4 °C. Data are mean ± S.E. from 15 to 35 cholangiocytes in each experimental group. *, $p < 0.05$ compared with corresponding −secretin values (Student's *t* test).

with those reported for other hormone-regulated transporters, such as aquaporin 2 and GLUT 4 (24–26). It is important to mention that minor levels of cross-contamination (about 10%) between plasma and microsomal membrane fractions were observed, but this did not significantly affect the calculations. Thus, the stoichiometric nature of this relationship further supports a redistribution of preexisting AQP1 water channels from an unidentified intracellular pool (associated with microsomal membranes) to cholangiocyte plasma membrane in response to secretin.

The fact that the secretin-induced relocation of AQP1 as well as the increased cholangiocyte P_f was disturbed by two perturbations (Figs. 3 and 4) that block secretin-induced exocytosis in cholangiocytes (10) (*i.e.* treatment with colchicine and exposure to low temperature) is consistent with the view that a microtubule-dependent exocytic insertion of AQP1-containing vesicles mediates the secretin-induced water permeability increase in cholangiocytes. Similar observations have been described for the vasopressin-mediated increase in water permeability in the kidney, which is also dependent on the integrity of the microtubular network (27). Our results also agree with observations in renal proximal tubule cells indicating that microtubules are involved in the insertion of AQP1 into plasma membranes (28).

In conclusion, the results of this study suggest that secretin facilitates osmotic water transport in cholangiocytes by inducing the microtubule-dependent targeting of vesicles containing

AQP1 water channels to the plasma membrane. We propose that this pathway provides a molecular mechanism accounting for the ability of secretin to stimulate ductal bile secretion.

Acknowledgments—We thank P. Tietz, B. Vroman, and J. Tarara for advice and assistance and D. Lubinski for secretarial help.

REFERENCES

1. Marinelli, R. A., and LaRusso, N. F. (1996) in *Seminars in Liver Disease* (Rothschild, M. A., Berk, P. D., and Gollan, J. L., eds), Vol. 16, pp. 221–229, Thieme Medical Publishers, Inc., New York
2. Boyer, J. L. (1996) *Am. J. Physiol.* **270,** G1–G5
3. Roberts, S. K., Yano, M., Ueno, Y., Pham, L., Alpini, G., Agre, P., and LaRusso, N. F. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 13009–13013
4. Yano, M., Marinelli, R. A., Roberts, S. K., Balan, V., Pham, L., Tarara, J. E., de Groen, P. C., and LaRusso, N. F. (1996) *J. Biol. Chem.* **271,** 6702–6707
5. Nielsen, S., Smith, B. L., Christensen, E. I., Knepper, M. A., and Agre, P. (1993) *J. Cell Biol.* **120,** 371–383
6. Nielsen, S., Smith, B. L., Christensen, E. I., and Agre, P. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 7275–7279
7. Farouk, M., Vigna, S. R., McVey, D. C., and Meyers, W. C. (1992) *Gastroenterology* **102,** 963–968
8. Buanes, T., Grotmol, T., Landsverk, T., and Raeder, M. G. (1988) *Gastroenterology* **95,** 417–424
9. Veel, T., Buanes, T., Grotmol, T., Engeland, E., and Raeder, M. G. (1990) *Acta Physiol. Scand.* **139,** 603–607
10. Kato, A., Gores, G. J., and LaRusso, N. F. (1992) *J. Biol. Chem.* **267,** 15523–15529
11. Bradbury, N. A., and Bridges, R. J. (1994) *Am. J. Physiol.* **267,** C1–C24
12. Knepper, M. A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 6255–6258
13. Ishii, M., Vroman, B. T., and LaRusso, N. F. (1989) *Gastroenterology* **97,** 1236–1247
14. Alpini, G., Phillips, J. O., and LaRusso, N. F. (1994) in *The Liver: The Biology and Pathobiology* (Arias, I. M., Boyer, J. L., Fausto, N., Jakoby, W. B., Schachter, D. A., and Shafritz, D. A., eds) Vol. 34, pp. 623–653, Raven Press, New York
15. Tietz, P. S., Alpini, G., Pham, L., and LaRusso, N. F. (1995) *Am. J. Physiol.* **269,** G110–G118
16. Tietz, P., Pham, L., and LaRusso, N. F. (1995) *Biochemistry* **34,** 15436–15443
17. Udenfriend, S., Stein, S., Bohlen, P., Dairman, W., Leimgruber, W., and Weigele, M. (1972) *Science* **178,** 871–872
18. Beaufay, H. Y., and Berthet, J. (1974) *J. Cell Biol.* **61,** 188–200
19. Denker, B. M., Smith, B. L., Kuhajda, F. P., and Agre, P. (1988) *J. Biol. Chem.* **263,** 15634–15642
20. Macey, R. I., and Farmer, R. E. L. (1970) *Biochim. Biophys. Acta* **211,** 104–106
21. Nielsen, S., Chou, C. L., Marples, D., Christensen, E. I., Kishore, B. K., and Knepper, M. A. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 1013–1017
22. Yamamoto, T., Sasaki, S., and Fushimi, K. (1995) *Am. J. Physiol.* **268,** C1546–C1551
23. Breyer, M. D., and Ando, Y. (1994) *Annu. Rev. Physiol.* **56,** 711–739
24. Marples, D., Knepper, M. A., Christensen, E. I., and Nielsen, S. (1995) *Am. J. Physiol.* **269,** C655–C664
25. Cushman, S. W., and Wardzala, L. J. (1980) *J. Biol. Chem.* **255,** 4758–4762
26. Piper, R. C., Hess, L. J., and James, D. E. (1991) *Am. J. Physiol.* **260,** C570–C580
27. Bourguet, J., Hugon, J. S., Valenti, G., and Svelto, M. (1988) *Comp. Biochem. Physiol.* **90A,** 669–672
28. Elkjaer, M., Birn, H., Agre, P., Christensen, E. I., and Nielsen, S. (1995) *Eur. J. Cell Biol.* **67,** 57–72